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Carcino-Embryonic Antigen Activity in Urine of Patients with Bladder Carcinoma

Clinical Evaluation of Carcino-Embryonic Antigen, II.

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Summary: This paper deals with the study of CEA levels in urine from healthy control subjects, of patients with bladder carcinoma or previous bladder carcinoma, and other groups of patients. In 53% of the patients with bladder carcinoma and without urinary infection, urinary CEA was increased. There were no false positive results. It is concluded that urinary CEA is a valuable aid in the detection of malignant tumour growth in the bladder. The results are compared with several tumour parameters, e.g. tumour size, stage of disease and histological parameters.

Evidence is presented that CEA in infected urine is not produced by bacteria and that, like soluble vaginal excretory products, this substance has antigenic groupings in common with CEA from bladder tumours.

Carcino-embryonales Antigen im Urin von Patienten mit Blasenkrebs
Klinische Bedeutung des carcino-embryonalen Antigens, 2. Mitteilung

Zusammenfassung: Die Konzentration des carcino-embryonalen Antigens (CEA) im Urin von Gesunden, von Patienten mit Blasenkrebs oder von Patienten, welche vorher Blasenkrebs hatten und von anderen Gruppen von Patienten wurde bestimmt. In 53% der Fälle von Patienten mit Blasenkrebs, aber ohne Harnwegsinfektion, waren die CEA-Werte erhöht. Bei Patienten, die mit Erfolg behandelt wurden, gab es keine erhöhten CEA-Werte. Daraus läßt sich schließen, daß die CEA-Bestimmung im Urin eine wertvolle Hilfe bei der Diagnostik von malignen Prozessen in der Blase darstellen kann. Die Ergebnisse wurden mit verschiedenen Parametern verglichen, z. B. Tumorgröße, Krankheitsstadium und histologischem Befund. CEA, welches in infiziertem Urin gemessen wird, wird nicht von Bakterien produziert, hat aber antigene Gruppen mit dem CEA aus Blasentumoren gemein.

Introduction

Carcino-embryonic antigen (CEA) was first described by *Gold & Freedman* (1). Studies on urinary carcino-embryonic antigen levels were not undertaken until 1972. In two interesting reports, *Hall* et al. (2, 3) presented data indicating a possible correlation between increased amounts of urinary CEA and bladder carcinoma. CEA[1]) levels in urine from healthy female subjects, however, showed widely varying and sometimes very high levels. The same group extended their studies of male patients and reported elevated values (> 35 µg/l) in 52 per cent of 45 patients (4). This was a lower incidence than that in their original study (2), covering 30 male patients. In a recent report (5) the same group extended their original work. The latter report indicated values < 35 µg/l as the normal range for females, which is quite different from the range (< 110 µg/l) used in a previous study. *Guinan* et al. (6) reported that the mean CEA level in the urines of 24 patients with bladder carcinoma was significantly higher than that of normal subjects.

The above mentioned authors remarked that more data are needed before the clinical value of CEA assays in urine can be definitely established. This study was started in order to extend available information on the clinical value of CEA measurements in the urine of patients with bladder carcinoma. An attempt was made to correlate several parameters of the tumour with urinary CEA levels. In addition, some experiments

[1]) Abbreviation: CEA = carcino-embryonic antigen.

were performed to gain more insight into the origin of the substance which is measured in the radio-immuno-assay for CEA in the urine of patients with bladder carcinoma.

Materials and Methods

Unless stated otherwise, urines were collected during cystocopy. Part of the samples of each urine was used to make cultures. The other part of the samples was then dialysed for four hours against phosphate buffered saline (50 mmol/l phosphate, 0.1 mmol/l K_2EDTA; pH 7.4), using cellulose acetate tubing. The dialysed portions were stored frozen until assay of CEA. Urinary cultures were grown on gel plates within a few hours of collection. In a number of cases the organisms were identified. Urinary infection was considered to be present if more than 10^8 colonies /l were found. CEA was measured in duplicate by the procedure described in a previous paper. Assays were performed in dialysed urines, undiluted and after dilution 1:2 with phosphate buffered saline (7). Extracts were made of bacterial cultures of urine samples. Bacterial colonies were removed from the plates and powdered in a "microdismembrator" (Braun, Melsungen, Germany) at low temperature (cooling in liquid nitrogen). The complete procedure has been described elsewhere (8). The powder was dissolved in the urine of healthy control subjects, containing less than 20 µg/l CEA. In the solution, thus prepared, CEA was measured. Vaginal and cervical discharges from women who were clinically free of carcinoma, were mixed with the urine of control subjects (< 20 µg/l CEA) for two hours at room temperature. The mixture was centrifuged for 10 minutes at 3.000 r.p.m.

Control subjects

In order to establish normal CEA values, CEA was estimated in the urines of 78 healthy subjects, 41 male and 37 female. In these cases midstream instead of catheter urine was taken. The ages varied between 20 and 61 years (male) and between 18 and 59 years (female). Three of the male subjects used medication. Thirteen of the female subjects used oral contraceptives and three used other medication.

Patients

The patients could be divided in 4 groups:

1. 78 patients, 60 male and 18 female, suffering from bladder carcinoma. Their ages varied between 42 and 93 years. The classification of their disease was established according to criteria of the Union Internationale contre le Cancer (9).
2. 134 patients, 110 male and 24 female, who had previously suffered from bladder carcinoma and showed no clinical evidence of the disease after treatment. The ages varied from 28 to 93 years.
3. 4 female patients with carcinomas of organs other than the bladder, i.e. 3 with cervical carcinoma and one with pyelum carcinoma. They had invasive tumour growth into the bladder as confirmed by cystoscopy but no infection of the urinary tract.
4. 19 patients who had an infection of the urinary tract but were not suffering from carcinoma, either at the time of urine collection or previously.

In all cases urinary CEA was estimated without knowledge of the clinical status of the subjects involved. Diagnosis was assessed and treatment given without reference to the CEA values. Therapeutic results were assessed and recorded independently of the results of the CEA assays.

Results

CEA levels in urine

Healthy controls

The results of CEA estimates in the urines of 41 males and 37 females are shown in figure 1, column I and II. Age, use of medication, length of menstrual cycle or time of urine collection in relation to the menstrual cycle had no demonstrable influence on the data. None of the subjects had a urinary infection. One woman, whose CEA level in midstream urine was 87 µg/l, allowed us to collect urine again, but with a catheter. Contrary to the midstream urine, the catheter urine contained less than 30 µg/l CEA.

Patients with bladder carcinoma

Figure 1 (column IV and V) shows the results of urinary CEA measurements in 53 patients without and 25 patients with urinary infection. Patients of both groups had evidence of bladder carcinoma. Of the 53 patients without urinary infection, 22 out of 41 men and 6 out of 12 women showed a urinary CEA excretion higher than 30 µg/l. There was no indication that parameters such as age, sex, time of diagnosis and previous treatment influenced the results.

Patients with previous bladder carcinoma

Urinary CEA was measured in 113 patients without urinary infection and 21 patients with urinary infection. None of these patients showed evidence of bladder carcinoma at the time of the investigation. The data are presented in figure 1 (column VI). Of these subjects, 92 had a normal bladder wall at the time of the urine collection; 18 showed a bladder with one or more papillomes, i. e. non-invasive bladder carcinoma and 3 appeared to have a radiation ulcus. Figure 1 (column VII) gives the results in 21 cases with a urinary infection.

Patients with primary carcinoma of other organs

Three patients (with cervical carcinoma) had CEA levels above 30 µg/l, the patient with pyelum carcinoma had a CEA level below 30 µg/l.

Patients with urinary infection only

Figure 1 (column III) shows the CEA levels in the urines of 19 patients with urinary infection. These patients never had evidence of any carcinoma.

Relation of urinary CEA to several parameters

Size of the tumour

In 45 patients with bladder carcinoma but without urinary infection the results of CEA estimation in urine were considered in relation to the diameter of the tumour, which was roughly measured at cystocopy. The diameter is supposed to be representative of the part of the tumour which is in contact with the bladder lumen. The data are listed in table 1.

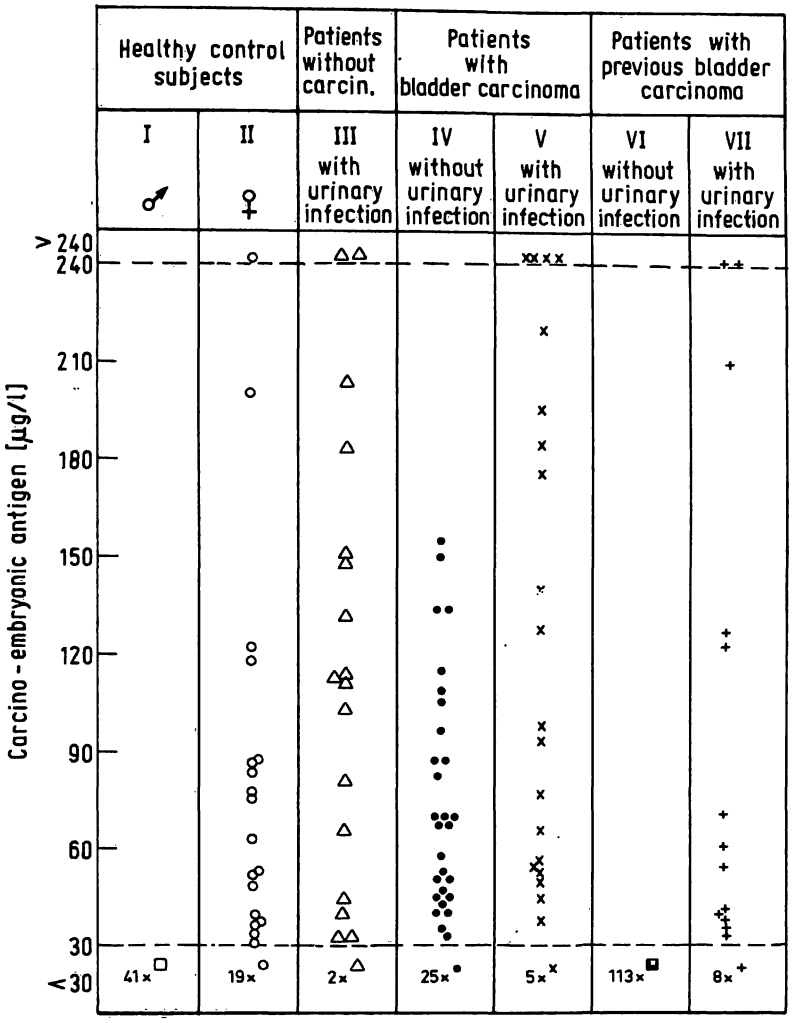

Fig. 1. Urinary CEA levels in patients and normal subjects.

Tab. 1. Relation of urinary CEA to tumour diameter.

Diameter of the tumour	CEA (µg/l)		
	> 100	30–100	⩽ 30
> 5 cm	7	12	10
3–5 cm	–	3	5
< 3 cm	–	3	5

Tab. 2. Relation of urinary CEA to stage of disease.

Stage of the disease	CEA (µg/l)		
	> 100	30–100	< 30
T_4	3	11	10
T_3	3	5	5
T_2	1	3	7
T_1	–	2	2

Stage of disease

Table 2 lists several ranges of CEA levels according to the clinical stage (9) of disease in 52 patients.

Histology

In 34 patients with bladder carcinoma but without urinary infection, histological findings were compared with the result of the nearest CEA estimation. The results are shown in table 3.

Additional experiments

We considered the possibility that elevated CEA levels in infected urine and in urine of female controls might be caused by interference in the CEA assay of excreted bacterial products and contamination of urine by vaginal or cervical secretion, respectively. Urinary extracts of homogenized bacteria, prepared as described under "Materials and Methods", were assayed for CEA. The bacteria studied were *E. coli, Enterococcus* and

Tab. 3. Histological parameters of bladder carcinoma in relation to urinary CEA.

	Histology	CEA (μg/l)		
		> 100	30–100	⩽ 30
Type	transitional cell carcinoma	4	7	14
	squamous cell carcinoma	–	–	1
	undifferentiated carcinoma	1	3	4
Papillary	+	3	3	8
	–	2	7	11
Differentiation	none	1	3	4
	poor	1	–	3
	moderate	2	6	7
	well	1	1	5
Polymorphism	slight	–	2	3
	moderate	2	4	5
	severe	3	4	11
Depth of invasion	none	–	–	1
	lamina propria	3	4	9
	muscularis	2	5*)	7
	non-classifiable	–	1	1
Inflammatory infiltrate	none	–	2	1
	slight	3	2	4
	moderate	1	2	8
	severe	1	4	6

*) One of these patients had a tumour, growing outside through the bladder wall.

Proteus. All extracts showed CEA levels below 30 μg/l. Non-infected urines with CEA levels below and above 30 μg/l were left standing covered at room temperature for one day. The samples were then tested for CEA and bacterial content. In some cases cultures were positive. No changes in CEA levels were observed. Extracts in urine of vaginal and cervical secretions obtained from two healthy females (see Materials and Methods) were analysed for CEA. The results are shown in table 4. Other experiments showed that proteinuria does not influence the CEA levels.

Tab. 4. Influence of vaginal and cervical secretion on urinary CEA.

Extract	CEA (μg/l)
Extract a.	> 300
1:2	138
1:5	55
1:10	31
Extract b.	> 300
1:2	> 300
1:10	109
1:20	56

Discussion

Concerning the question of whether urinary CEA should be expressed as the CEA/creatinine ratio or as CEA per 24-hour volume, we offer the following comment. CEA in urine is produced by the urothelium and very probably not excreted through the kidneys. This can be concluded from the fact that patients with other carcinomas and high blood CEA levels have normal urinary CEA. For this reason a relation between urinary CEA and creatinine excretion could not be expected to exist. Furthermore, it is obvious that a CEA/creatinine ratio cannot be calculated in cases in which the urinary levels of CEA are below the borderline of detection. Another argument is that the retention period of the urine in the bladder does not seem to influence the concentration of urinary CEA. This may be concluded from the data in table 5, showing nearly identical CEA levels for a 3 hour and a 15 minute period. For clinical evaluation, we have therefore used CEA levels measured as μg/l in urines independently of the time of collection.

Like *Hall* et al. (2, 3) we found a wide range of urinary CEA values in female controls. Our results differ from theirs in that a larger percentage (43%, column II in fig. 1) of values above 35 μg/l was found when measuring midstream samples. This range may constitute a problem in the interpretation of urinary CEA levels in female patients with bladder carcinoma. The data from figure 1, column VI, demonstrate that the use of catheter urine solves this problem. Another conclusion which can be drawn from figure 1 (column I and VI) is that the upper limit of normal is 30 μg/l. It should be noted that, if the standard inhibition curve permitted measurement in the 20–30 μg/l range (7), values between 20–30 μg/l were found in several cases in both groups. The borderline of 30 μg/l seems therefore real. Infection of the urinary tract leads to a rise of urinary CEA in even a larger percentage of cases than does bladder carcinoma (see fig. 1, columns III and VII). In this respect it should be borne in mind that urinary infections can be associated with very high CEA levels. We therefore agree with *Hall* et al. (2) that the possibility of urinary infection has to be excluded before CEA levels are used for diagnostic purposes. We disagree with these authors that the problem of false positive values can be largely eliminated by the use of midstream urine. The possibility of false negatives in catheter urines must be accounted for (fig. 1, column IV). Comparison with the data of *Guinan* et al. (6) is difficult

Tab. 5. Relation of urinary CEA to the retention period of urine in the bladder.

Patient	Period of retention	CEA (μg/l)
♂ K	180 minutes	48.5
♂ K	15 minutes	50.0
♂ F	180 minutes	24.0
♂ F	15 minutes	24.5

since these authors report mean CEA values in different groups of urine samples but without distinguishing between the presence and absence of infections or between the presence and absence of tumours. Their conclusion that urinary infection causes elevated CEA levels which are not as high as in the case of a tumour, is not sustained by our results. A possible explanation might be the use of perchloric acid extraction by these authors, which causes breakdown of CEA (7).

The above restrictions need some comment. *Hall* et al (3) suggested on the basis of some experiments that the occurrence of positive CEA values in female controls might be caused by contamination of the urine with vaginal and cervical secretions. Since no experimental details were given and urines tested for CEA, if turbid, are cleared by centrifugation, we considered it useful to estimate the fraction of secretions soluble in urine for CEA activity. The results outlined in table 4 confirm the suggestion of *Hall* et al. Additional support lies in the positive and negative CEA value found in midstream and catheter urine of the same female control (see Results). The concentration values given in table 4 may not represent the real concentrations of the substances in the secretion which exert CEA activity. (The same may apply to CEA measured in urines from bladders with a tumour or an infection). Increased as well as normal CEA values were observed with the common bacteria which can cause urinary infection. Elevated CEA levels in infected urine therefore cannot be related to one type of bacterium. Our results (see additional experiments) warrant the conclusion that the bacteria themselves do not produce CEA. The only explanation for the increase in urinary CEA in infection seems to be that irritation or changing of the bladder mucus by the bacteria leads to release of CEA or substances with CEA-like activity. The fact that CEA activity in infected urines, urines with vaginal excretions and urines from bladders with a tumour show parallelism to the standard curve, leads to the conclusion that the active substances have antigenic groupings in common. We tried to absorb-out the antiserum with extracts of vaginal and cervical secretions in urine and found no residual activity against colonic CEA. A more precise description of the identity of the above substances cannot be given.

A relation between CEA levels and tumour diameters might be expected. Statistically, urinary CEA levels are indeed related to the tumour diameter ($p = 0.035$) (tab. 1) but not to the stage of disease ($p = 0.36$) (tab. 2) or to any of the histological parameters ($p > 0.50$ for any of the parameters) (tab. 3). Increased urinary CEA values are not only related to the bladder tumours, since invasive growth of other tumours into the bladder may also cause increased values.

Urinary CEA levels in patients with bladder carcinoma were elevated in 53% of the cases without infection (fig. 1, column IV). In none of 113 patients successfully treated for bladder carcinoma were increased CEA values ever obtained. In other words: there were no false positives results (fig. 1, column VI). Non infiltrating carcinomas of the bladder (so called papillomata) of such patients did not cause increased values. In view of these results, urinary CEA estimates are clinically valuable for detection of tumour growth in the bladder. To obtain diagnostically reliable CEA values in females a catheter specimen is indispensable. Since this is problematic in healthy females, urinary CEA estimates for screening purposes will be confined in practice to male subjects. (fig. 1, column I, IV and VI). The follow-up findings will be presented in a subsequent publication.

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